find that elasticity becomes comparable to viscous behavior at collagen concentrations of 5mg/ml. By varying electrostatic interactions in the system, we demonstrate the role they play in conferring elasticity to collagen solutions.

2623-Pos Board B609
Molecular Mechanics of Type I Collagen
Stephanie L. Holdener, Heather M. Harper, William G. Matthews.
Understanding the development of extracellular matrix (ECM) mechanics is of great importance for those interested in cell differentiation and tissue engineering. Collagen is responsible in large part for the mechanical properties of the ECM, and this is especially true for the collagenous tissues formed from type I collagen fibrils. Studies of the flexibility of collagen at the molecular scale have been underway for some time. However, detailed knowledge of how the flexibility of type I collagen molecules varies within the molecule has not been experimentally investigated to date. The collagen molecules largely are triple helicies, though non-helical domains are plentiful. Do these non-helical domains result in 'hinges', where the flexibility dramatically increases locally? Or are these small perturbations from the persistence length determined by the helical domains? To address these questions, we have developed a technique that maps the deviation from a straight path along the molecular length as imaged by atomic force microscopy. This deviation reflects the local flexibility of the molecule. We then are correlating these maps between molecules, seeking to assign local flexibilities to the known amino acid sequence.

2624-Pos Board B610
Experimental Validation of Free Energy Landscape Reconstructions from Non-Equilibrium Single-Molecule Pulling Experiments
Abhilash Vincent, Amar Nath Gupta, Krishna Neupane, Hao Yu, Michael Woodside.
Force spectroscopy techniques have been widely used to understand the structural and elastic properties of proteins and nucleic acids, examining in particular the conformational changes associated with folding and unfolding transitions. The free energy landscapes governing these transitions are important for understanding folding but difficult to measure experimentally. It has been demonstrated that non-equilibrium single-molecule force spectroscopy measurements can be used to reconstruct the profile of the equilibrium free energy landscape, by employing an elegant extension of the Jarzynski equality. Although this method has been previously applied to experiments and simulations, it has not yet been validated through quantitative comparisons to equilibrium measurements of the free energy landscape profile. Here, we are validating this method through force-extension measurements conducted on DNA hairpins exhibiting distinct, sequence-dependent folding landscapes. We find that the free energy profiles obtained from non-equilibrium pulling measurements agree well with the landscapes obtained from equilibrium measurements conducted under constant force. We also investigate the application of the non-equilibrium method to systems with multiple folding intermediate states, through force-extension measurements of an adenine riboswitch aptamer with 5 distinct states.

2625-Pos Board B611
Unstressed Off-Rate Determination from Multiple Bond Rupture
Vijay K. Gupta, Charles D. Eggleton.
Using dynamic force spectroscopy to measure the kinetic off-rates of intermolecular bonds currently requires the isolation of single molecules. This requirement arises in part because no tractable analytic method for determining kinetic off-rates from the rupture of a large number of bonds under dynamic forces is currently available. We introduce a novel method for determining the unstressed off-rate from dynamic force spectroscopy experiments involving a large number of bonds. Using both the Dembo and Bell models we show that the unstressed off-rate calculated using the proposed method is in good agreement with the prescribed unstressed off-rate used in Monte-Carlo simulations off multiple bond dynamic force spectroscopy experiments given initial number of bonds (50-500) and loading rate 10^3-10^6 pN/s. The results indicate that the error in proposed method decreases as the number of bonds increases.

2626-Pos Board B612
Crack Propagation on Networks: A Model for Protein Unfolding
Adam M.R. de Graff, Gareth Shannon, Daniel W. Farrell, Philip M. Williams, Michael F. Thorpe.
The manner in which proteins respond under an applied force is of direct biological significance, as a complete understanding of the mechanical, regulatory and signaling properties of many proteins depend not only on their native state conformations, but also on the nature of partially unfolded intermediate states that become populated under an applied force.
The inability of molecular dynamics simulation to reach timescales probed experimentally has led some to use coarse-grained elastic network models to study unfolding properties. While computationally efficient, these models have been limited to the study of the native state.
Building on a recently developed method called geometric targeting [1], we use a constraint-based, all-atom representation to generate complete unfolding pathways. The model uses a simplified potential to maintain proper stereochemistry and represents hydrogen bonds, salt bridges and hydrophobic interactions as breakable inequality constraints. By iteratively increasing the extension of a protein and breaking constraints according to a set of simple rules, we demonstrate that the simple and intuitive model of protein unfolding as crack propagation on a constraint network is sufficient to capture the unfolding pathways and experimentally observed intermediates of a diverse set of proteins far from their native states.
[1] Farrell, D. W., K. Speranskiy, and M. F. Thorpe. 2010. Generating stereochemically acceptable protein pathways. Proteins: Structure, Function, and Bioinformatics 78:2908-2921.

2627-Pos Board B613
Computational Modeling Predicts Novel Collagen Conformations During Mechanical Loading
Jonathan W. Bourne, Peter A. Torzilli.
Collagens contribute to the mechanical strength of many tissues throughout the body and are generally resistant to ezymatic degradation. As structural proteins, collagens often experience *in vivo* mechanical forces such as expansion and contraction of blood vessels and tension on tendons and ligaments. Collagen crosslinking, which enhances the strength of these structural networks, occurs during tissue development and aging. While much is known about the structure of collagen, there is a paucity of data describing how mechanical force transmitted across these crosslinks affects molecular conformation. We hypothesized that mechanical force applied perpendicular to the long axis of the collagen triple helix will result in bending and microunfolding of the triple helix structure. To test this we used Steered Molecular Dynamics to model the conformation of a collagen peptide when subjected to perpendicular forces. *In silico* loading predicted that the collagen peptide had minimal resistance to bending, and exhibited increased curvature with no distinct disruption of the characteristic triple helix at low forces. As force increased, we observed that the helix began to fail and underwent a microunfolding event, where a loop pulled out from the complex. This local triple helix disruption was predicted to occur below covalent bond failure strength, suggesting that alternative molecular conformations occur within the molecule as structures are loaded before the onset of structural failure. We speculate that these changes may represent nano-damage to the structure and be a mechanism for energy storage and dissipation. Furthermore, these predicted conformational changes would precede macroscopic damage mechanisms.

2628-Pos Board B614
Effect of Rapamycin on Filamentous Fungal Cell Walls
Usha Sripathineni, Bill J. Moss, Liming Zhao, Robert W. Roberson, David Schaefer, Mark R. Marten.
Filamentous fungi are widely used in the bioprocess industry to produce a variety of products resulting in billion dollar returns. Often times in industrial bioprocesses, fungi experience nutrient limitation, and we hypothesize that autophagy, a nutrient starvation response, is induced. We further hypothesize that autophagy leads to changes in the mechanical properties of filamentous fungal cell walls, resulting in thinner, weaker and stiffer walls. We test this hypothesis here, using rapamycin (an immunosuppressant drug) to gratuitously induce autophagy in the model fungi *Aspergillus nidulans*. Atomic force microscopy (AFM) is used to assess the mechanical properties of the cell wall, electron microscopy is used to assess wall thickness and a novel fragmentation assay is used to determine relative tensile strength of the culture. We will report on these studies and how they support our hypotheses.

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2629-Pos Board B615
Nanocluster Beacon (NCB): A DNA-Silver Nanocluster Probe that Fluoresces upon Hybridization
Hsin-Chih Yeh, Jaswinder Sharma, Jason J. Han, Jennifer S. Martinez, James H. Werner.
Oligonucleotide-templated silver nanoclusters (DNA/Ag NCs) are an emerging set of fluorophores that have seen applications in cellular imaging and chemical/biological detection. Recently we discovered the red fluorescence emission of DNA/Ag NCs could be enhanced more than 500 fold when brought into close proximity to a guanine-rich DNA sequence (Yeh et al., Nano Letters, 10 (8): 3106-3110, 2010). Based on this finding, we developed a new type of molecular probe (termed NanoCluster Beacon, NCB) that fluoresces upon target DNA binding. Compared to molecular beacons, NCBs require only a single labeling step and do not rely on F [[Unable to Display Character: ő]]rster energy transfer as fluorescence switching mechanism. Moreover, there is no need to remove the silver nanocluster precursors (Ag+ and BH4-) used during